Table 3. Principal contaminants detected in snowy and great egret eggs collected at the Salton Sea in 1993. In calculating the geometric mean a value of one half the detection limit was used for samples in which no measurable quantity was detected. Geometric means are presented if ≥ 50% of samples contained measurable quantities of a given contaminant. No mean is presented if < 50% of samples contained measurable quantities of a given contaminant.

Species	Location	Egg Id	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p, DDEp µg/g ww	PCB µg/g ww	Toxaphene µg/g ww
Snowy Egret	Whitewater	2	< 505	4.8	0.06	4.3	0.14	< .05
		3	< 996	4.6	< 0.05	2.6	< .05	< .05
		4	501	4.7	< 0.1	1.3	< .05	< .05
		10	< 504	5	0.19	2.1	< .05	< .05
		15	< 502	4.6	0.02	2.1	< .05	< .05
		16	< 505	8	0.2	4.1	0.35	< .05
		18	< 502	4.1	0.14	1.4	0.07	< .05
		19	< 504	4.1	0.1	2.7	< .05	0.06
		20	< 506	5.5	0.07	6.1	< .05	< .05
		21	0.76	7.2	< 0.02	1.7	< .05	< .05
	Mean			5.07	0.04	0.3		
	Minimum			3.5	< 0.1	1.7		
	Maximum			8.32	0.2	4.1		
	Wht/Water	1	< 1	6.1	0.06	2.2	0.12	1.1
		6	< 502	6.6	0.08	4.5	0.5	< .05
Great Egret		8	504	6.6	0.25	50	0.75	0.63
		10	2.1	6.1	0.4	6.1	1.1	0.76
		11	0.67	7.9	0.26	7.2	0.47	1.3
	Poe Road	1	< 5	7.8	0.11	17	0.8	2.6
		2	< 0.57	6.9	0.42	16	0.7	2.2
		3	1.28	6.8	0.37	14	0.6	2.1
		5	0.78	9.9	0.45	15	2.5	0.77
	Poe Road Delta	3	0.90	7.1	0.16	11	4.6	1.4
	Mean		0.55	7.14	0.28	13.11	0.90	0.68
	Minimum		ND	6.1	0.08	4.5	0.42	ND
	Maximum		2.1	9.9	0.45	50	4.6	2.6

eggs collected contained embryos that could be examined in detail and all 40 eggs collected in 1993 were sub mitted for chemical analysis. Each of these 1993 nests was observed for nest proficiency (defined as producing a full clutch of viable eggs) and those results are presented below.

The principal contaminants observed in each of the stilt eggs collected in 1992 and 1993 are presented in Tables 4 and 5, respectively. On a population basis, the 1992 stilt eggs contained a geometric mean of 6.60 µg/g DW selenium (range 3.74 to 14.2) and the 1993 stilt eggs contained a geometric mean of 5.82 µg/g DW selenium (range 3.67 to 8.96). These levels indicate the Salton Sea black-necked stilt populations are, on average, at the Level of Concern for bird hazard [Bureau of Reclamation 1993 Internal Memorandum to NIWQP Manager regarding Predicted Selenium Effect Levels for Kendrick and Middle Green River Remediation (Irrigation Drainage) 4 pp.].

The stilt eggs contained geometric mean concentrations of 0.47 µg/g DW boron in 1992 and 1.09 µg/g DW boron in 1993 (Tables 4 and 5). Both geometric mean concentrations are below the 3 µg/g DW threshold value for boron affects to duckling growth (Smith and Anders 1989).

The stilt eggs also contained geometric mean concentrations of 2.02 µg/g WW DDE in 1992 and 2.48 DDE in 1993. In 1993, when 40 stilt eggs were sampled, a single stilt egg was found to contain 23 µg/g WW DDE, a value twice as high as the 12.0 µg/g WW maximum observed when 84 stilt eggs were analyzed in the 1988-90 NIWQP irrigation drainwater study of the Salton Sea area (Setmire et al. 1993). Adverse effects occur in many fish-eating birds at <10 ppm DDE, but the level of DDE associated with reproductive failures in black-necked stilts is not specifically known.

One stilt egg collected in 1992 (Egg ID 11) had signs of embryotoxicity that included hemorrhaging of the throat and had a selenium content of 8.3 µg/g DW (Table 4). One stilt egg collected in 1993 at Johnson Drain at the north end of the Salton Sea (Egg ID Johnson Drain 36) had a lower mandible slightly smaller relative to the upper, and had a selenium content of 5.9 µg/g DW (Table 5). Neither of these malformations by themselves are considered selenium terata, as selenium induced deformities typically include multiple malformations of the eyes, bills and limbs.

The fate of the black-necked stilt nests monitored at the Salton Sea in 1993 is presented in Table 6. The black-necked stilt nest proficiency data set was analyzed to determine the proportion of nests with reproductive impairment by having ≥1 eggs that failed to hatch. Of the 37 nests observed, 27 were full term nests that were not preyed upon, abandoned or destroyed. However, four of those full term nests were excluded from further analysis because their fate was ambiguous in that at least one egg from each nest was known to have hatched, but the fate of the remaining eggs could not be determined (these four nests are indicated in Table 6 as having hatched 1+ eggs). One full term nest (Elmore Ranch 1) was observed to initially contain a full clutch of eggs and, although the fate of two eggs in that nest was unknown, the nest did contain one egg that failed to hatch. Two other nests each contained one failed-to-hatch egg (Davis Road 10 and Hazard Tract 18). Therefore, three of the 23 full term nests had ≥1 fail-to-hatch eggs, or 13% of the black-necked stilts nests studied were affected by hatching failure.

Table 4. Principal contaminants in black-necked stilt eggs collected around the Salton Sea in 1992. In calculating geometric mean a value of one half the detection limit was used for samples in which no measurable quantity was detected. Geometric means are presented if ≥ 50% of samples contained measurable quantities of a given contaminant. No mean is presented if < 50% of samples contained measurable quantities of a given contaminant.

Black-necked Stilt Eggs Collected in 1992							
Egg Id	Percent Moisture	Percent Lipid	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p,p'-DDE µg/g ww	Toxaphene µg/g ww
1	73	14	0.4	8.1	0.05	1.9	<0.05
2	76	<1.3	<0.5	4.0	0.11	3.5	<0.05
3	74	14	<0.5	5.7	<0.01	3.6	<0.05
4	71	12	0.64	5.9	<0.01	2.3	<0.05
5	75	12	1.29	4.7	<0.01	0.62	<0.05
6	75	14	0.51	5.1	0.05	1.9	<0.05
7	73	10	<0.5	8.3	0.04	2.1	<0.05
8	76	10	<0.5	6.71	<0.01	3.4	<0.05
9	76	8	<0.5	5.1	0.12	1.7	<0.05
10	78	17	0.76	6.29	0.03	1.16	<0.05
11	76	12	<0.5	8.3	0.03	1.1	<0.05
12	68	17	<0.5	8.7	0.09	2.1	<0.05
13	75	16	<0.5	6.5	0.01	0.69	<0.05
14	74	15	<0.5	5.1	0.06	3.8	<0.05
15	72	11	<0.5	6.8	0.04	1.2	<0.05
16	75	6	0.9	4.8	<0.01	0.36	<0.05
17	76	12	0.93	7.3	<0.01	1.7	<0.05
18	78	12	0.7	5.7	0.04	0.92	<0.05
19	70	18	<0.5	7.9	0.06	2.4	0.9

Black-necked Stilt Eggs Collected in 1992

Egg Id	Percent Moisture	Percent Lipid	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p,p'-DDE µg/g ww	Toxaphene µg/g ww
20	74	14	0.76	7.9	<0.01	5.7	<0.05
21	72	6	0.94	5.5	0.02	1.4	<0.05
22	74	15	<0.5	12.9	0.05	0.8	<0.05
23	74	10	<0.5	6.8	<0.01	0.9	<0.05
24	73	14	0.69	5.7	0.04	2.2	<0.05
25	74	12	<0.5	7	0.03	1	<0.05
26	75	9	<0.5	13.6	0.04	2.7	<0.05
27	72	12	1.17	6.2	0.04	2	<0.05
28	70	13	<0.5	11.3	0.04	1.8	<0.05
30	75	12	<0.5	4.6	0.06	2.6	<0.05
31	75	12	2	6.9	0.07	2.7	<0.05
32	69	14	0.6	6	0.04	2.6	<0.05
33	72	13	1.3	5.2	0.06	2.2	<0.05
34	69	14	0.5	6.1	0.05	3.1	<0.05
35	66	13	0.7	6.1	0.02	1.1	<0.05
36	63	14	0.7	7.3	0.06	5.8	3.2
37	5	39	<0.5	14.2	0.05	6	<0.05
38	26	32	<0.5	4.3	0.09	5.9	<0.05
39	0.1	36	1.2	5.6	0.01	7.5	<0.05
Mean			0.47	6.60	0.03	2.02	
Minimum			<0.05	3.74	<0.01	0.36	
Maximum							

Table 5. Principal contaminants in black-necked stilt eggs collected around the Salton Sea in 1993. In calculating geometric mean, a value of one half the detection limit was used for samples in which no measurable quantity was detected. Geometric means are presented if ≥ 50% of samples contained measurable quantities of a given contaminant. No mean is presented if < 50% of samples contained measurable quantities of a given contaminant.

Black-necked Stilt Eggs Collected in 1993								
Egg Id	Location	Percent Moisture	Percent Lipid	Boron ug/g dw	Selenium ug/g dw	Dieldrin ug/g ww	p,p'-DDE ug/g ww	Toxaphene ug/g ww
1	Davis Road	73	15	2.26	9.0	<0.01	5.9	<0.05
2	Davis Road	70	14	<0.5	5.9	0.06	3.1	<0.05
3	Davis Road	70	8	2.19	5.7	0.01	0.96	<0.05
4	Davis Road	71	15	1.77	7.0	0.04	8.7	0.32
5	Davis Road	71	14	1.85	6.5	0.05	2.9	<0.05
6	Davis Road	70	13	2.89	7.1	<0.03	0.3	<0.05
7	Davis Road	72	12	1.69	7.2	<0.01	0.05	<0.05
8	Davis Road	69	16	1.77	6.7	0.01	2.4	<0.05
9	Davis Road	70	15	1.91	6.3	0.03	2.9	<0.05
10	Davis Road	69	18	1.17	6.5	0.02	1.7	<0.05
11	Davis Road	68	17	0.96	5.2	0.03	2.6	<0.05
12	Hazard Tract	71	15	1.4	4.7	0.03	1	<0.05
13	Hazard Tract	71	15	0.98	4.6	0.17	9.2	<0.05
14	Hazard Tract	71	14	<0.5	5.7	0.07	3.1	<0.05
15	Hazard Tract	72	12	1.4	5.6	0.04	1.1	<0.05
16	Hazard Tract	68	15	1.08	4.4	0.04	1.9	<0.05
17	Hazard Tract	67	16	1.96	7.1	0.07	1.6	<0.05
18	Hazard Tract	70	17	0.58	4.3	0.03	1.4	<0.05
19	Hazard Tract	69	14	1.97	4.9	0.06	3	<0.05
20	Hazard Tract	70	17	1.1	4.3	0.06	2.1	<0.05
21	Hazard Tract	70	15	<0.5	4.2	0.1	2.8	<0.05
22	Hazard Tract	64	19	<0.5	6.0	0.03	8.2	<0.05
23	Hazard Tract	70	14	<0.5	3.7	0.02	1.1	<0.05

Black-necked Stilt Eggs Collected in 1993

Egg Id	Location	Percent Moisture	Percent Lipid	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p,p'-DDE µg/g ww	Toxaphene µg/g ww
24	Hazard Tract	72	13	<0.5	7.7	0.02	1.5	<0.05
25	Hazard Tract	72	13	1.3	5.4	0.03	3	<0.05
26	Davis Road	70	14	1.7	4.0	0.06	3.8	<0.05
28	Davis Road	72	13	2.87	6.9	<0.01	2.6	<0.05
29	Davis Road	70	15	2.71	4.7	0.06	2	<0.05
30	Davis Road	72	13	1.89	7.5	<0.01	2.3	<0.05
31	Hazard Tract	71	13	<0.5	6.9	0.3	7.2	1.6
32	Hazard Tract	70	11	1.65	7.9	0.04	1.3	<0.05
33	Hazard Tract	59	25	1.57	4.4	0.04	2.3	<0.05
34	Hazard Tract	62	19	1.5	3.9	0.04	1.9	<0.05
35	Hazard Tract	71	18	0.85	6.6	0.37	8.7	2.4
36	Johnson Drain	70	14	1.9	4.8	0.02	0.7	<0.05
37	Johnson Drain	70	13	2.2	5.9	0.027	1.1	<0.05
38	Barth Road	71	10	1.5	7.7	0.15	2.1	<0.05
39	Barth Road	69	15	0.95	6.7	0.02	1.4	<0.05
40	Barth Road	71	10	[illegible]	6.8	0.05	1.5	<0.05
41	Hazard Tract	71	11	1.87	8.0	0.05	1.5	<0.05
42	Hazard Tract	70	14	2.48	6.4	0.08	1.9	<0.05
43	Hazard Tract	64	17	<0.5	6.6	0.39	6.3	1.6
44	Hazard Tract	39	32	1.20	4.5	0.06	2.5	0.41
45	Hazard Tract	65	14	<0.5	5.8	0.31	5	<0.05
Mean				1.09	5.82	0.040	2.48	
Minimum				0.25	3.67	<0.01	0.65	
Maximum				2.89	8.96	0.39	23	

The number of stilt eggs collected from nests in 1992 and 1993 considered at increased risk to hatching failure because of selenium toxicity is presented in Figure 3. In both years of the study, 95% of the stilt eggs sampled contained ≥4.2 ppm selenium, meaning the nests those eggs came from were four times more likely to fail than if the eggs contained less than 4.2 ppm DW selenium (Ohlendorf et al. 1993, Skorupa 1994).

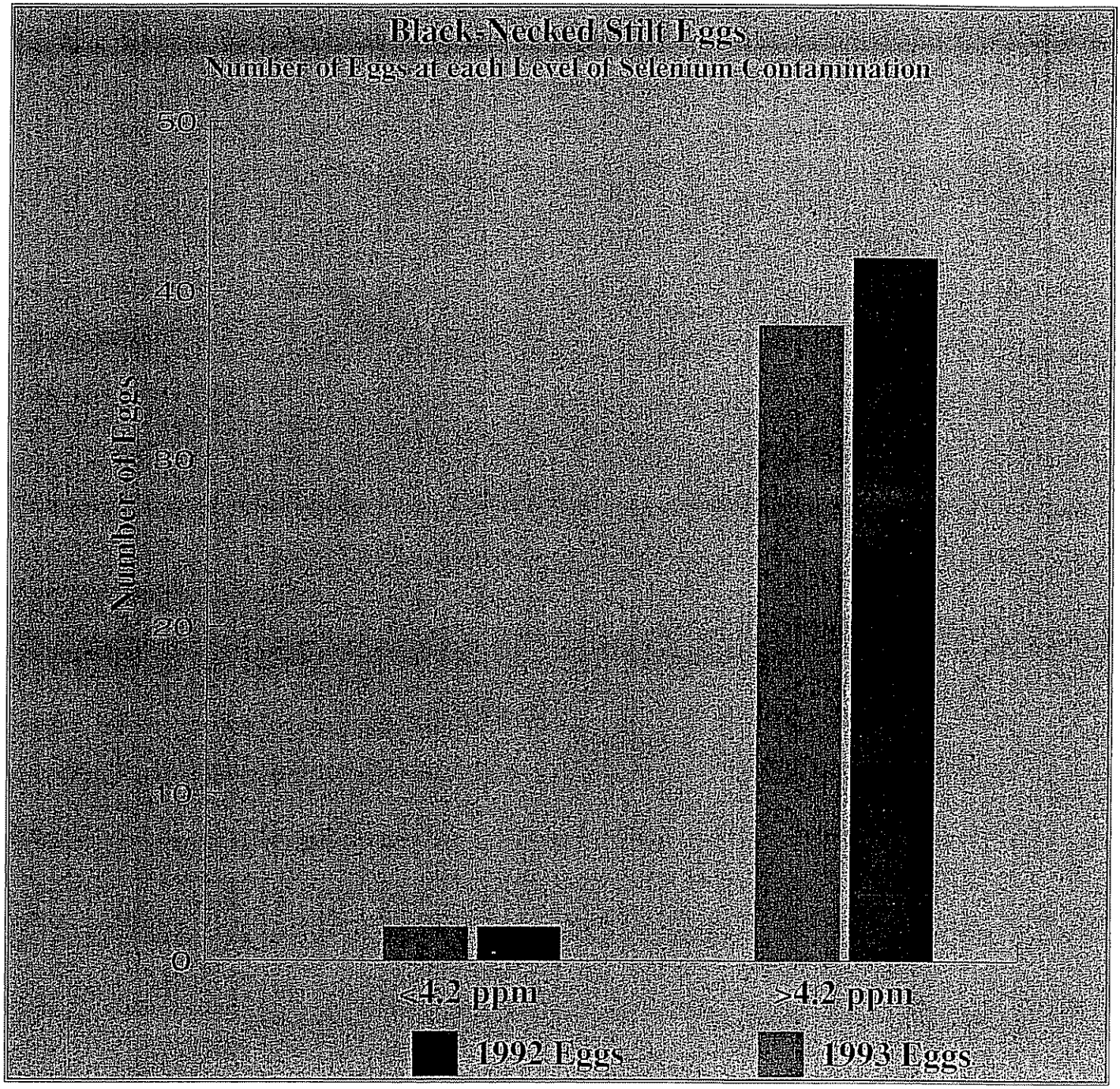

Figure 3. Number of black -necked stilt eggs collected in 1992 and 1993 at different levels of reproductive risk relative to selenium concentrations in the egg. Eggs containing 4.2 ppm or more selenium are about four times more likely to be from reproductively impaired nests than eggs with less than 4.2 ppm selenium (Ohlendorf et al. 1993, Skorupa 1994).

Normally, only 8.9% of stilt nests with less than 4.1 ppm DW selenium in their eggs have ≥1 fail-to-hatch eggs, or, conversely, 91.1% of normal nests are unaffected by fail to hatch eggs (USFWS 1995). When the Salton Sea black-necked stilt productivity (87.0% unaffected nests) is compared to normal productivity, it was calculated the Salton Sea population had 4.5% reproductive depression when compared to stilts in selenium-normal environments [(91.1-87.0)/91.1 =0.045]. Although the sample size was not adequate for statistical evaluation, this amount of reproductive depression in the Salton Sea black-necked stilt population is attributed to the selenium concentrations in the eggs because: 1) the reproductive success calculations were based only on data from full-term nests that did not fail due to predation, disturbance or abandonment, and 2) selenium

Therefore, there is an apparent small (4.5%) effect in black-neck stilt reproduction that is consistent with known exposure-response data, and there is good reason to consider it biologically real. However, due to the small magnitude of effect, and the low statistical power of the sample size of 23 nests, the possibility that the result represents pure chance cannot be rejected. For example, a chi-square comparing the null expectation of 2.0 impaired nests (and 21.0 unimpaired, i.e., 8.9% impaired, the background rate) against the observed ratio of 3 impaired nests and 20 unimpaired nests yields a chi-square value of <1.0 (d.f.=1), which does not approach statistical significance at the 5% level. Based on the rate of impairments observed, it has been calculated that a sample size of about 225 full term nests would need to be monitored in order to statistically verify a 4.5% reduction in nesting proficiency. Monitoring that sample size of black-necked stilts is not realistic for any given year at the Salton Sea. However, it could realistically be achieved over several nesting seasons.

The avian reproductive hazard in the Salton Sea area was reviewed in a presentation at the 1994 Salton Sea Symposium (Skorupa 1994). Because there is a close correlation between waterborne selenium and egg selenium in nonmigratory species of water birds, and a close correlation between egg selenium and toxic effects, toxicity thresholds for avian eggs can be used to estimate toxicity thresholds in water and the diet. These relationships and estimates have been independently field validated in two recent studies of avian exposure to selenium (USFWS 1995), therefore, the general toxicological relationships documented for the San Joaquin Valley also appear to apply to data from the Salton Sea. For example, water-to-egg selenium ratios for black-necked stilts documented for the Salton Sea fall on the same regression line as data from Kesterson Reservoir and the Tulare Basin (Dr. Joseph Skorupa, personal communication, 1995). Using that information, it could be predicted that approximately 11.9% (or, when rounded to the nearest whole number of nests for this sample set, three) of Salton Sea stilt nests would be affected by hatching failure. In fact, 13% (i.e., three) of Sea stilt nests were observed to be affected by hatching failure in this study. Therefore, black-necked stilts nesting at the Sea appear to display the same selenium exposure response described for birds in other localities (Skorupa 1994, USFWS 1995). This demonstrates again the taxonomic and geographic robustness of the selenium toxicity thresholds currently established for birds, and that a large amount of predictive information about bird reproductive hazard can be obtained simply by knowing the concentration of selenium in the environment and the egg.

This study demonstrated that black-necked stilts are a good species to monitor for selenium- induced effects at the Salton Sea because: 1) no other contaminant was observed in their eggs at concentrations high enough to be a known reproductive impairment, and 2) the biological response of the stilts to the selenium exposure is apparently consistent with exposure-response data, although the sample size of Salton Sea stilt nest success data is small relative to desirable statistical power. Therefore, it would be desirable to continue monitoring black-necked stilts in the Salton Sea area to describe selenium effects on the area's waterbirds, and more clear conclusions can be reached within a few breeding seasons.

Table 6. Fate of black-necked stilts nests (and associated egg contamination) located around the Salton Sea in 1993. Shaded selenium values indicate the selenium concentration in that egg was in the hatchability depression range (5.1-20 ppm) for individual recurverostrid eggs (Skorupa et al. 1992).

Nest Fate and Egg Contaminant Information of Black-Necked Stilts Nesting Around Salton Sea, 1993							
Nest Location	Nest Id	Clutch Size	Number Hatched	Number Fail to Hatch	Number Young Dead	Nest Fate	Selenium µg/gdw2
Johnson Drain	2	3	1	0	0	Hatched	4.8
	3	3	1+	0	0	Hatched	5.9
Elmore Ranch	1	3	0	1+	0	Unknown	7.7
	2	3	1+	0	0	Hatched	6.7
	3	3	1+	0	1	Hatched	6.8
Davis Road	1	3	3	0	1	Hatched	9.0
	2	3	3	0	0	Hatched	5.9
	3	3	3	0	0	Hatched	5.7
	4	3	3	0	0	Hatched	7.1
	5	3	0	0	0	Predated	6.5
	6	3	3	0	0	Hatched	7.1
	7	3	3	0	0	Hatched	7.2
	8	3	3	0	0	Hatched	6.7
	9	3	3	0	0	Hatched	6.3
	10	3	2	1	0	Hatched	6.5
	11	3	3	0	0	Hatched	5.4
	26	3	0	0	0	Predated	4.0
	27	3	0	0	0	Predated	
	28	3	3	0	0	Hatched	6.9
	29	3	3	0	0	Hatched	4.7
	30	3	3	0	0	Hatched	7.5
Hazard Tract	12	3	0	2	0	Abandoned	4.7
	13	3	3	0	0	Hatched	4.6

Nest Fate and Egg Contaminant Information of Black-Necked Stilts Nesting Around Salton Sea, 1993

Nest Location	Nest Id	Clutch Size[1]	Number Hatched	Number Fail to Hatch	Number Young Dead	Nest Fate	Selenium µg/gdw[2]
	14	3	3	0	0	Hatched	5.7
	15	3	3	0	0	Hatched	5.6
	16	3	0	3	0	Abandoned	4.4
	17	3	3	0	0	Hatched	7.4
	18	3	2	1	0	Hatched	4.3
	19	2	2	0	0	Hatched	4.9
	20	3	0	0	0	Destroyed	4.3
	21	3	0	0	0	Predated	4.2
	22	3	0	3	0	Abandoned	6.0
	23	3	0	0	0	Predated	4.7
	24	3	3	0	0	Hatched	7.7
	25	3	3	0	0	Hatched	5.4
	31	3	3	0	0	Hatched	6.9
Gas Road	32	2	0	2	0	Abandoned	7.9

[1]Clutch size after removal of one egg
[2] Chemical content of single egg removed from clutch

Study 3. Desert Pupfish Study Using Sailfin Molly Surrogates

Sailfin mollies were trapped in 13 agricultural drains. The principal contaminants detected in sailfin mollies and desert pupfish collected in drains around the Salton Sea in 1994 are presented in Table 7. Geometric mean boron concentrations in the fish ranged from 3.3 to 28.0 µg/g DW, with the maximum concentration (32.3 µg/g) observed in mollies from Trifolium 12 Drain. It is not possible to compare these boron results with those of the NIWQP Detailed Study (Setmire et al. 1993) because in that study the boron reporting limit in sailfin molly tissues was 18 µg/g. The results are difficult to evaluate further because there are no boron criteria or effect levels for fish. Geometric mean DDE concentrations in mollies ranged from 0.03 to 0.93 µg/g WW, with the higher DDE concentrations seen in fish collected from the Vail 7 Drain (0.51 µg/g DDE), the R Drain (0.52 µg/g DDE), and the Trifolium 23 Drain (0.93 µg/g DDE). These concentrations of DDE are not hazardous to the fish themselves, but some approach the National Academy of Sciences 1 µg/g organochlorine pesticide threshold for protection of fish-eating birds (NAS 1972). Also, the elevated DDE concentrations probably

used to determine if particular drainages are contributing more bioavailable organochlorines to the ecosystem than others.

In the case of W Drain, where both sailfin mollies and desert pupfish were collected and submitted for analysis, there was general agreement in contaminant levels between the two species, particularly in selenium where mollies contained 5.6 µg/g DW and desert pupfish contained 5.4 µg/g DW. Therefore, it seems that sailfin mollies are reasonably good indicators for desert pupfish selenium contamination loads.

Sailfin mollies from 11 of the 13 drains and the desert pupfish from one of the drains contained geometric mean concentrations between 3 to 6 ppm DW selenium and therefore were at the Level of Concern for warmwater fishes [Bureau of Reclamation 1993 Internal Memorandum to NIWQP Manager regarding Predicted Selenium Effect Levels for Kendrick and Middle Green River Remediation (Irrigation Drainage) 4 p.]. Sailfin mollies in two other drains (Avenue 76 and Trifolium 18) contained geometric mean concentrations of 6.4 and 10.2 µg/g selenium DW, respectively, and so were over the toxicity threshold (>6 ppm) for warmwater fish reproductive hazards. Selenium hazards to desert pupfish are not precisely known, but they may have a sensitivity similar to that of the fathead minnow, another warmwater fish in the same family, Cyprinidae (Dr. Steve Hamilton, National Biological Service, personal communication 1995). Juvenile fat head minnows exhibit growth inhibition at whole body concentrations of 6 to 8 µg/g DW selenium (Bennett et al. 1986, and Ogle and Knight 1989), and there is a significant increase in edema and lordosis (curved spine) in larval fathead minnows when adults were exposed to selenium water at concentrations as low as 10 µg/l. Lemly (1993) concluded that 4 µg/g DW selenium be considered the toxic effect threshold for the overall health of and reproductive vigor of freshwater fish. Therefore, the desert pupfish is apparently at reproductive risk in many of the drains where they are known to occur.

Finally, fish collected from all drains exceeded either the Level of Concern (2-6 ppm DW) or Toxicity Threshold (>6 ppm DW) for dietary criteria, indicating that besides the risk to the fish themselves, the fish also present a risk to organisms that consume them.

Table 7. Geometric mean (range) concentration of principal contaminants in sailfin mollies and desert pupfish from drains around the Salton Sea in 1994. Lightly shaded selenium values indicates the concentrations in fish were in the Level of Concern (3-6 ppm dw), and dark shaded values indicate concentrations in fish were above the Toxicity Threshold (>6 ppm dw) for warmwater fish reproductive hazards (BOR 1993).

Sailfin Mollies Collected in 1994					
Location	Sample Size	Boron µg/g dw	Selenium µg/g dw	Dieldrin µg/g ww	p,p'-DDE µg/g ww
Avenue 76	3	3.7 3.1-4.7	6.4 6.2-6.7	0.022 <0.01-0.030	0.37 0.27-0.43
Avenue 81	3	9.3 5.7-11.7	2.4 1.8-3.3	<0.01	0.23 0.16-0.33
Niland 1	3	6.0 5.1-7.3	4.8 4.1-5.6	<0.01	0.12 0.11-0.14
Poe Road	3	6.1 5.5-7.0	5.0 4.4-5.4	<0.01 0.12-0.15	0.13
R-Drain	2	4.7 3.4-6.1	3.1 3.1-3.2	<0.01 0.24-0.59	0.52
Trifolium 12	3	28.0 24.2-32.3	3.6 3.5-3.8	<0.01	0.064 0.06-0.066
Trifolium 13	1	5.4	4.6	No data	No data
Trifolium 18	3	11.3 11.0-11.8	10.2 8.9-11.7	<0.01	0.03 0.029-0.031
Trifolium 19	1	4.4	5.3	<0.01	0.13
Trifolium 22	3	4.3 4.2-4.6	5.7 5.6-6.3	0.049 0.048-0.051	0.35 0.34-0.37
Trifolium 23	1	3.3	4.2	0.082	0.93
Vail 7	3	16.7 9.5-22.2	5.2 5.0-5.6	0.025 0.015-0.031	0.44 0.31-0.51
W Drain	3	6.2 5.3-7.1	5.6 5.3-5.6	<0.01	0.24 0.21-0.26
W Drain Pupfish	2	8.8 8.6-9.1	5.4 5.1-6.1	<0.01	0.17 0.14-0.19

CONCLUSIONS

The amount of eggshell thinning (up to 12%) observed in black-crowned night herons nesting at the Salton Sea in 1993 indicates that species is likely to be experiencing reproductive depression related to egg failures. Embryonic malformations were observed in 29% of the snowy egret and great egret embryos examined in detail. A variety of defects were observed, including the unusual malformation of a twin embryo joined at the body but with a single head, but none of these malformations were considered typical selenium-induced terata. The deformities observed could be related to the multiple kinds of contaminants observed in the egret eggs, but these kinds of synergistic effects are poorly understood. Chemical analysis of the egret embryos indicated the egg selenium content ranged from 3.5-9.9 µg/g DW -- levels that put the birds at risk to lowered productivity but unlikely to produce observable rates of teratogenicity in the small number of egret eggs examined. Therefore, neither the kinds of deformities observed in the egrets nor the associated levels of selenium in their eggs indicate that selenium-induced terata is occurring in egrets nesting at the Salton Sea area. However, the egret eggs also exhibited high levels of DDE with a geometric mean of 6.33 µg/g WW in the snowy egrets and 13.11 µg/g WW in the great egrets. These levels of DDE approach and exceed the amount (8 µg/g) associated with reduced reproductive success in black-crowned night herons (Custer et al. 1983, and Henny et al. 1984). An additional concern is that some egrets contained surprisingly higher levels of DDE and toxaphene in this study than in another study of contaminants in egrets from the Salton Sea in 1985. Apparently, high levels of these persistent contaminants are still available to some of these birds. Setmire et al. (1993) identified DDE contamination at all trophic levels (including resident species) in the Salton Sea ecosystem. In fact, it was detected in 99% of the samples analyzed, and concentrations in biota were correlated with trophic level. That study concluded that resident species of birds in the Imperial Valley are likely to experience reproductive impairment as a result of the DDE contamination. Other sources of DDE, including possible sources in Mexico, are potentially available to migratory species. However, a recent study by Mora (1997) indicates that there is no clear evidence for increased bioaccumulation of DDE in migratory species while wintering in Mexico. The limited data suggest that bioaccumulation is similar in Mexico and the Southwestern United States. The reported declines in colonial nesting bird success at the Salton Sea is likely to be related to the high levels of multiple contaminants in these fish-eating birds, particularly organochlorines.

The black-necked stilt study indicates that this species is likely to be experiencing selenium-induced reproductive depression. Nesting proficiency of Salton Sea area stilts was 4.5% lower than that reported for stilts with low selenium exposure (USFWS 1995), with 13% of the full term stilt nests affected by having at least one egg that failed to hatch. The geometric mean selenium content of the stilt eggs (6.60 µg/g DW in 1992 and 5.82 µg/g DW in 1993) places the stilts at a four-times greater risk to reproductive depression due to egg mortality than if they had selenium levels below 4.1 ppm (Skorupa 1994). The stilts at Salton Sea did not exhibit selenium-induced terata, but the likelihood of observing embryo deformation at those eggs selenium concentrations and the samples sizes of this study would be very low (Dr. Joseph Skorupa, personal communication, 1995). Therefore, when assessing the hazard of selenium to stilts at the Salton Sea, the most appropriate type of investigation is a nesting proficiency study with chemical analysis of one egg from each nest.

One aspect that needs to be considered when evaluating the data for black-necked stilts is their level of sensitivity to selenium toxicity. Stilts are moderately sensitive to the reproductive effects of selenium toxicity as compared to ducks which are considered sensitive to these effects (Skorupa, J. P., S.P. Morman, J.S. Sefchick-Edwards 1996 Internal Memorandum to the NIWQP regarding Guidelines for Interpreting Selenium Exposures of Biota Associated with Nonmarine Aquatic Habitats, 74 pp.). While the effect measured here was small, an effect was detectable in a moderately sensitive species. This raises concerns about the potential for reproductive impairment in more sensitive species that nest in the Salton Sea ecosystem.

The sailfin mollie study indicated that the endangered desert pupfish is probably also at risk to reproductive hazards from selenium. In addition, the sailfin mollie data indicate that fishes in the agricultural drains are

summarize the management implications of this study, the reproductive depression in birds due to both
enium and DDE, hazards to the endangered pupfish, and levels of selenium in fish as a dietary food item
ve emerged as the most serious concerns for fish and wildlife resources in the Salton Sea area. The biologi-
hazards relative to persistent contaminants in the Salton Sea area are now more clearly understood and the
formation indicates which species and endpoints are most relevant to efforts at improving and monitoring the
Salton Sea contaminant issues, with respect to NIWQP responsibilities.

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APPENDIX 1

Analytical Chemistry Methods for Bird Tissues

Chemical analyses for inorganic compounds in bird tissues were performed at Research Triangle Institute (Research Triangle Park, NC). Elements analyzed included arsenic, aluminum, boron, barium, beryllium, cadmium, chromium, copper, iron, magnesium, manganese, mercury, molybdenum, nickel, lead, selenium, strontium, vanadium, and zinc. Tissue samples were prehomogenized using a food processor and a portion of the sample was freeze-dried for determination of moisture content and ground through a 100-mesh screen with a mill. Using a CEM microwave oven, 0.5 g of the freeze-dried samples were heated in a capped vessel in the presence of 5 ml of Baker Instra-Analyzed nitric acid for three minutes at 120 watts, then three minutes at 300 watts, and finally for 35 minutes at 450 watts. Vessel caps were rinsed into the vessel with additional nitric acid and the uncapped vessel was returned to the microwave and heated until the volume remaining was less than 1 ml. Samples were then diluted to 5 ml with laboratory pure water and centrifuged to precipitate the suspended material. Samples then underwent inductively coupled plasma spectroscopy (ICP) analysis (USEPA 1987b, Dahlquist and Knoll 1978) using a Leeman Labs Plasma Spec I sequential or ES2000 simultaneous spectrometer. Samples that underwent graphite furnace or cold vapor atomic absorption were homogenized as described above for ICP analysis. These samples were similarly heated in a microwave, however the duration of microwave heating at 450 watts reduced to 15 minutes. Residues produced were then diluted to 50 ml with laboratory pure water. Graphite furnace atomic absorption measurements for arsenic and selenium (USEPA 1984) were made with a Perkin-Elmer Zeeman 3030 or 4100ZL atomic absorption spectrometer. Cold vapor atomic absorption measurements for mercury (USEPA 1984) were conducted using SnC^{14} as a reducing agent with a Leeman PS200 Hg Analyzer.

Chemical analyses for organic compounds in bird tissues were performed at Mississippi State Chemical Laboratory (Starkville, MS). Organic analytes analyzed included organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs), cis-nonachlor, and delta BHC. Tissue samples were thoroughly mixed with anhydrous sodium sulfate and soxhlet extracted (USEPA 1986) with hexane for seven hours. Extracts were concentrated via rotary evaporation and concentrated todryness for lipid determination. After weighing, samples were dissolved in petroleum ether and extracted four times using acetonitrile saturated with petroleum ether. Residues were partitioned into petroleum ether which was then washed, concentrated, and transferred to a glass chromatographic column containing 20 g of Florisil. The column was eluted with 200-ml 6% diethyl ether and 94% petroleum ether followed by 200-ml 15% diethyl ether and 85% petroleum ether. The second fraction was concentrated to the appropriate volume for quantification by packed or capillary column electron capture gas chromatography using a Varian 6000/6500 or Varian 3600 gas chromatograph. The first fraction was concentrated and transferred to a silicic acid chromatographic column for the additional clean-up required to separate PCB's from the other OC's. Three fractions were eluted from the silicic acid column, and each was concentrated to the appropriate volume for quantification by packed or megabore column electron capture gas chromatography using a Varian 6000/6500 or Varian 3600 gas chromatograph.

Analytical Chemistry Methods for Fish Tissues

Chemical analyses for inorganic elements in fish tissues were performed at Hazleton Laboratories America, Inc. (Madison, WI). Analysis included quantification of the same elements as in the bird tissue analysis described above. Percent moisture was determined by weighing the sample in a tared aluminum dish then drying in an oven at 100 C for 12-18 hours until a constant weight was reached (AOAC 1990). Elemental analysis was conducted via ICP. Digestion was carried out in nitric acid in a microwave digester. Emission intensities were compared to series of identification standards using a Thermo Jarrell Ash ICAP 61E spectrometer, with the spectrometer program correcting for background and interfering elements. Mercury analyses were

conducted using cold vapor atomic absorption spectroscopy (USEPA 1984). Samples were digested using sulfuric and nitric acids, and the mercury was reduced with sodium borohydride for determination. Mercury concentrations were determined at wavelength of 253.7 nm using a Leeman Labs PS200 atomic absorption spectrophotometer with an MHS-20 hydride generation unit, with the signal compared to standard solutions. Arsenic and selenium analyses were conducted using graphite furnace atomic absorption spectroscopy (USEPA 1984) on a Perkin-Elmer Zeeman 5100 PC spectrophotometer. Samples were digested with nitric acid in a microwave digester. Arsenic was determined at 193.7 nm wavelength and selenium was determined at 196.0 nm wavelength. The nickel matrix modification was employed in the analysis, and standard additions were conducted when interferences were indicated. Organic analyses of the fish samples were also performed at Hazelton Laboratories America, Inc. These methods included the determination of OCs and PCBs, but did not include cis-nonachlor and delta BHC. Percent moisture was determined by weighing a 1-10 g of sample in a pre-weighed aluminum pan. Samples were dried in an oven at 105 C for 16 hours and allowed to cool in a desiccator before being weighed again (USEPA 1986). The following equation was used to calculate the percent moisture: [mass (g) pan + wet sample] - [mass (g) pan + dry sample] x 100/grams of sample = % moisture grams of sample. Spiking solutions are added to the samples after the tissues were ground. Pesticide spikes were added to a portion of the sample matrices and control spikes of 2,4,5,6-tetrachloro-m-xylene were used as a blank spike solution for all other samples. Tissue samples were then dried under a hood using anhydrous sodium sulfate. A soxhlet extractor was
used with methylene chloride to extract the desired fractions from the samples (USEPA 1986). The resulting extracts were then concentrated in a Kuderna-Danish apparatus to a volume of 5 ml on a hot water bath, then diluted to 10 ml with methylene chloride. One ml subsamples of this extract were used for lipid determination. Subsamples were placed in a pre-weighed aluminum pan and placed under a hood to evaporate the solvent. Pans were weighed again, and the following equation used to calculate percent lipid: [(weight (g) of pan + lipid) - weight (g) of pan] x 10 ml x 100/grams of sample = % lipid grams extracted. Five ml volumes of the remaining extract were injected on an ABC Laboratories Model 1002B Gel-Permeation Chromatography system for clean-up (USEPA 1990) using a column packed with 70 g of S-X3 Bio-beads with methylene chloride as the carrier solvent. This extract was again concentrated to 5 ml. Then 50 ml of hexane was added to the samples and they were concentrated for a third time to a 5 ml volume. Additional clean-up and separation of PCBs from the OCs was carried out in a silica gel column. The first fraction was eluted with petroleum ether and the second fraction was extracted using a mixture of 1% acetonitrile, 19% hexane and 80% methylene chloride. Both fractions were then concentrated using the Kuderna-Danish apparatus, followed by dilution with hexane and a repeat of the concentration step. The fractions underwent electron capture gas chromatography using a Hewlett-Packard 5890 gas chromatograph for quantification of individual constituents.

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